IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:Confirmation No. 7978
Serial No. 10/559,835	: Group Art Unit 1633
Takehisa Matsuda	: Attorney Docket No. 2005_1807A
Filed : March 8, 2006	: Examiner LEAVITT, MARIA GOMEZ

DECLARATION UNDER 37 CFR 1.132

(No.1)

Honorable Commissioner of Patents and Trademarks

Sir:

I, Kunio MATSUMOTO hereby declare that:

I was born in Nagano prefecture, Japan, in 1959;

I am a citizen of Japan and a resident of 2-9-3-303, Hiro-oka, Kanazawa City, Kanazawa 920-0031 JAPAN;

I graduated from Department of Biology, Faculty of Science, Kanazawa University, Japan in 1981.

I received my doctor degree on the study of "Analysis of intermolecular relationship in photosynthetic oxygen evolving complex" at Osaka University, Japan, in 1986;

I have worked as an Associate Professor of Osaka University in Japan from 1990-2007 and as a Professor of Kanazawa University from 2007 until now and have engaged in a study on hepatocyte growth factor and NK4;

I am one of the inventors for this application;

I have many reports relating to HGF and NK4. A part of my reports are as follows:

1: Matsumoto, K., and Nakamura, T.: Hepatocyte growth factor (HGF) as tissue organizer for organogenesis and regeneration. *Biochem. Biophys. Res. Commun.*, 239, 639-644, 1997.

2: Matsumoto, K., and Nakamura, T.: Mechanisms and significance of bifunctional NK4 in Cancer Treatment. *Biochem. Biophys. Res. Commun.*, 333: 316-327, 2005.

3: Matsumoto, K., Nakamura, T., Sakai, K., and Nakamura, T.: Hepatocyte Growth Factor and Met in Tumor Biology and Therapeutic Approach with NK4. *Proteomics*, 8: 3360-3370, 2008.

4: Date, K., Matsumoto, K., Shimura, Tanaka, H. H. M. and Nakamura, T.: HGF/NK4 is a specific antagonist for pleiotrophic actions of hepatocyte growth factor. *FEBS Lett.*, 420, 1-6, 1997.

5: Date, K., Matsumoto, K., Kuba, K., Shimura, H., Tanaka, M. and Nakamura, T.: Inhibition of tumor growth and invasion by a four-kringle antagonist (HGF/NK4) for hepatocyte growth factor. *Oncogene*, 17: 3045-3054, 1998.

6: Matsumoto, K., Kataoka, H., Date, K. and Nakamura, T.: Cooperative interaction between α-chain and β-chain of HGF on c-Met receptor confers ligand-induced receptor tyrosine phosphorylation and multiple biological responses. *J. Biol. Chem.*, 273, 22913-22920, 1998

7: Kuba, K., Matsumoto, K., Date, K., Shimura, H., Tanaka, M., and Nakamura, T.: HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppress tumor growth and metastasis in mice. *Cancer Res.*, 60: 6737-6743, 2000.

8: Tomioka, D., Maehara, N., Kuba, K., Mizumoto, K., Tanaka, M., Matsumoto, K., and Nakamura, T.: Inhibition of growth, invasion, and metastasis of human pancreatic carcinoma cells by NK4 in an orthotopic mouse model. *Cancer Res.*, 61: 7518-7524, 2001.

9: Maemondo, M., Narumi, K., Saijo, Y., Usui, K., Yahara, M., Tazawa, R., Hagiwara, K., Matsumoto, K., Nakamura, T., and Nukiwa, T.: Targeting angiogenesis and HGF function using an adenovirul vector expressing the HGF-antagonist NK4 for cancer therapy. *Mol. Therapy*, 5: 177-185, 2002.

10: Kikuchi, T., Maemondo, M., Narumi, K., Matsumoto, K., Nakamura, T., and Nukiwa, T.: Tumor suppression induced by intratumor administration of adenovirus vector expressing NK4, a 4-kringle antagonist of hepatocyte growth factor, and naïve dendritic cells. *Blood*, 100: 3950-3959, 2002.

11: Martin, T. A., Parr, C., Davies, G., Watkins, G., Lane, J., Matsumoto, K., Nakamura, T., Mansel, R. E., and Jiang, W. G.: Growth and angiogenesis of human breast cancer in a nude mouse tumour model is reduced by NK4, the HGF/SF antagonist. *Carcinogenesis*, 24: 1317-1323, 2003.

12: Manabe, T., Mizumoto, K., Nagai, E., Matsumoto, K., Nakamura, T., Nukiwa, T., Tanaka, M., and Matsuda, T.: Cell-based protein delivery system for the inhibition of the growth of pancreatic cancer: NK4 gene-transduced oral mucosal epithelial cell sheet. *Clin. Cancer Res.*, 9: 3158-3166, 2003.

13: Davies, G., Mason, M. D., Martin, T. A., Parr, C., Watkins, G., Lane, J., Matsumoto, K., Nakamura, T., and Jiang, W. G.: The HGF/SF antagonist NK4, reverses fibroblast- and HGF-induced prostate tumor growth and angiogenesis in vivo. *Int. J. Cancer*, 106: 348-354, 2003.

14: Wen, J., Matsumoto, K., Taniura, N., Tomioka, D., and Nakamura, T.: Hepatic gene expression of NK4, an HGF-antagonist/angiogenesis inhibitor, suppresses liver metastasis and invasive growth of colon cancer in mice. *Cancer Gene Therapy*, 11: 419-430, 2004.

15: Murakami, M., Nagai, E., Mizumoto, K., Saimura, M., Ohuchida, K., Inadome, N., Matsumoto, K., Nakamura, T., Maemondo, M., Nukiwa, T., and Tanaka, M.: Suppression of metastasis of human pancreatic cancer to the liver by transportal injection of recombinant adenoviral NK4 in nude mice. *Int. J. Cancer*, 117: 160-165, 2005.

16: Namiki, Y., Namiki, T., Yoshida, H., Date, M., Yashiro, M., Matsumoto, K., Nakamura, T., Yanagihara, K., Tada, N., Satoi, J., and Fujise, K.: Preclinical study of a "tailor-made"

combination of NK4-expressing gene therapy and gefitinib (ZD1839, Iressatrade mark) for disseminated peritoneal scirrhous gastric cancer. *Int. J. Cancer*, 118:1545-1555, 2006.

17: Ogura, Y., Mizumoto, K., Nagai, E., Murakami, M., Inadome, N., Saimura, M., Matsumoto, K., Nakamura, T., Maemondo, M., Nukiwa, T., and Tanaka, M.: Peritumoral injection of adenovirus vector expressing NK4 combined with gemcitabine treatment suppresses growth and metastasis of human pancreatic cancer cells implanted orthotopically in nude mice and prolongs survival. *Cancer Gene Ther.*, 13: 520-529, 2006.

18: Son, G., Hirano, T., Seki, E., Iimuro, Y., Nukiwa, T., Matsumoto, K., Nakamura, T., and Fujimoto, J.: Blockage of HGF/c-Met system by gene therapy (adenovirus-mediated NK4 gene) suppresses hepatocellular carcinoma in mice. *J. Hepatol.*, 45: 688-695, 2006.

19: Du, W., Hattori, Y., Yamada, T., Matsumoto, K., Nakamura, T., Sagawa, M., Otsuki, T., Niikura, T., Nukiwa, T., and Ikeda, Y.: NK4, an antagonist of hepatocyte growth factor (HGF), inhibits growth of multiple myeloma cells in vivo; molecular targeting of angiogenic growth factor. *Blood*, 109: 3042-3049, 2007.

20: Kishi, Y., Kuba, K., Nakamura, T., Wen, J., Suzuki, Y., Mizuno, S, Nukiwa, T., Matsumoto, K, and Nakamura, T. Systemic NK4 gene therapy inhibits tumor growth and metastasis of melanoma and lung carcinoma in syngeneic mouse tumor models. *Cancer Sci.*, 100: 1351-1358, 2009.

The experiments given below were conducted under my supervision.

Experiment

(1)Method

Experiment 1

Effect of angiostatin on HGF-induced DNA synthesis of rat hepatocytes in primary culture was examined.

Specifically, Hepatocytes were prepared from 7-week old male Sprague-Dawely rats by *in situ* perfusion of the liver with collagenase, as described elsewhere (Seglen P. O. *Methods Cell Biol.* 1976, 13, 29-83; Nakamura T., Tomita Y., and Ichihara A. *J. Biochem.* 1983, 94, 1029-10356; 19). The cells were cultured on plastic dishes coated with type I-collagen at a cell density of $1.2 \times 10^5/cm^2$ for the confluent culture and $0.3 \times 10^5/cm^2$ for the sparse culture. The cells were cultured in Williams' E medium supplemented with 5% (v/v) fetal bovine serum, 5 nM insulin, and 10 nM dexamethazone. After 24-hours of culture, HGF and varying concentrations of angiostatin were added to cultures of hepatocytes. The cells were further cultured for 20 h, and then pulse-labeled with 0.3 mCi/ml ^{125}I-deoxyuridine for 6 hours. The cells were washed twice with phosphate-buffered saline and once with trichloroacetic acid, then solubilized with 1 M NaOH. Radioactivity of ^{125}I-deoxyuridine incorporated into nuclei was measured using a γ-counter.

Angiostatin was purified from slastase-treated human plasminogen. Human recombinant HGF was purified from the conditioned medium of Chinese hamster ovary cells transfected with human HGF cDNA (Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) *Nature* 342, 440-443).

Experiment 2

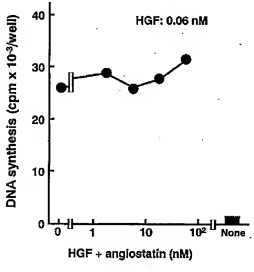
Effect of angiostatin on HGF-induced cell scattering of MDCK cells was examined. Specifically, MDCK (clone 3B) renal epithelial cells, a kind gift from Dr. R. Montesano (University of Geneva) were cultured in DMEM containing 10% fetal calf serum. MDCK cells were seeded on a 48-well plate at a density of 2.5 X 10^3 cells/well in DMEM containing 10% fetal calf serum, with or without HGF and angiostatin. The cells were cultured for 20 h, then photographed.

(2) Result

Experiment 1

The result is shown in Fig.1 below.

Fig.1

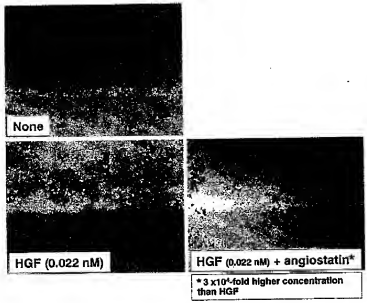


As is clear from Fig.1, HGF-induced DNA synthesis of rat hepatocytes was not inhibited by angiostatin at all.

Experiment 2

The result is shown in Fig.2 below.

Fig.2



As is clear from Fig.2, HGF-induced cell scattering of MDCK cells was not inhibited by angiostatin, although angiostatin was used in a concentration of 3×10^4-fold higher than that of HGF.

(3) Discussion

As a result, it is evident that angiostatin does not exhibit an antagonist activity against HGF.

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the

knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18U.S.C.1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: Oct. 7, 2009

Kunio MATSUMOTO